AD_____

Award Number: DAMD17-02-1-0507

TITLE: Regulation of Estrogen-Responsive Gene Expression and Tumor Suppression by Transcriptional Cofactors

PRINCIPAL INVESTIGATOR: Hua Xiao, M.D., Ph.D.

CONTRACTING ORGANIZATION: University of Nebraska Medical Center
Omaha, NE 68198-7835

REPORT DATE: May 2005

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2005	Final (1 May 2002 - 30 Apr 2005)

4. TITLE AND SUBTITLE
Regulation of Estrogen-Responsive Gene Expression and Tumor Suppression by Transcriptional Cofactors

5. FUNDING NUMBERS
DAMD17-02-1-0507

6. AUTHOR(S)
Hua Xiao, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Nebraska Medical Center
Omaha, NE 68198-7835

E-Mail: hxiao@unmc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)* TIP30 is a tumor suppressor that promotes apoptosis and inhibits angiogensis through the regulation of transcription. We previously found that TIP30 interacts with an ERα-interacting coactivator CIA (Coativator Independent of AF2). We propose to determine the effects of TIP30 and CIA on expression of ERα-responsive genes, such as c-Myc and Cyclin D1 in the first year. Now, we demonstrate that TIP30 and CIA are dynamically associated with transcription initiation and elongation complexes in response to estrogen. TIP30 overexpression represses ERα-mediated c-*myc* transcription, whereas TIP30 deficiency enhances *c-myc* transcription in the absence and presence of estrogen. Ectopic CIA cooperates with TIP30 to repress ERα-mediated c-*myc* transcription. Overexpression of CIA in ER-negative MDA-MB-231 cells induces apoptosis whereas overexpression of TIP30 does not. However, overexpression of both TIP30 and CIA in MDA-MB-231 cells effectively induce apoptosis. Therefore, our data suggest that TIP30 and CIA act as negative regulators to control transcription of genes including *c-myc* and cyclin D1 thereby suppressing tumor development. Thus, human Tip30 and CIA may represent new valuable diagnostic and therapeutic targets for anti-breast cancer therapy.

14. SUBJECT TERMS
Estrogen receptor, transcriptional repressor, apoptosis, c-myc

15. NUMBER OF PAGES
14

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction:

The growth and metastatic potential of breast cancer cells are regulated by estrogen, a ligand of the estrogen receptors (ERs) (1, 2). The ERs facilitate major effects of estrogen in cells through interactions with transcriptional cofactors to regulate transcription of ER-targeting genes. We previously identified two human cofactors TIP30 and CIA (Coativator Independent of AF2) that can specifically regulate ERα-mediated transcription. TIP30, also called CC3 or Htatip2, is a tumor suppressor that can promote apoptosis and inhibits angiogensis, through the regulation of transcription (3-5). TIP30 interacts with an ERα-interacting coactivator CIA (6,7). Therefore, we hypothesized that overexpression of both TIP30 and CIA will inhibit transcription of ER-target genes such as c-Myc and Cyclin D1 and overexpression of TIP30 and CIA in ER-negative MDA-MB-231 breast cancer cells suppresses cell growth. We proposed to determine the effects of TIP30 and CIA on expression of ERα-responsive genes, such as c-Myc and Cyclin D1 in the first year. Now, we show that TIP30 overexpression represses ERα-mediated c-*myc* transcription, whereas TIP30 deficiency enhances *c-myc* transcription in the absence and presence of estrogen. Ectopic CIA cooperates with TIP30 to repress ERα-mediated c-*myc* transcription. Using chromatin immunoprecipitation (ChIP) assays (8), we demonstrate that TIP30 and CIA are distinct cofactors that are dynamically associated with transcription initiation and elongation complexes in response to estrogen. Both TIP30 and CIA are recruited to the *c-myc* gene promoter by liganded ERα in the second transcription cycle. Overexpression of TIP30 and CIA in ER-negative MDA-MB-231 breast cancer cells suppresses cell growth.

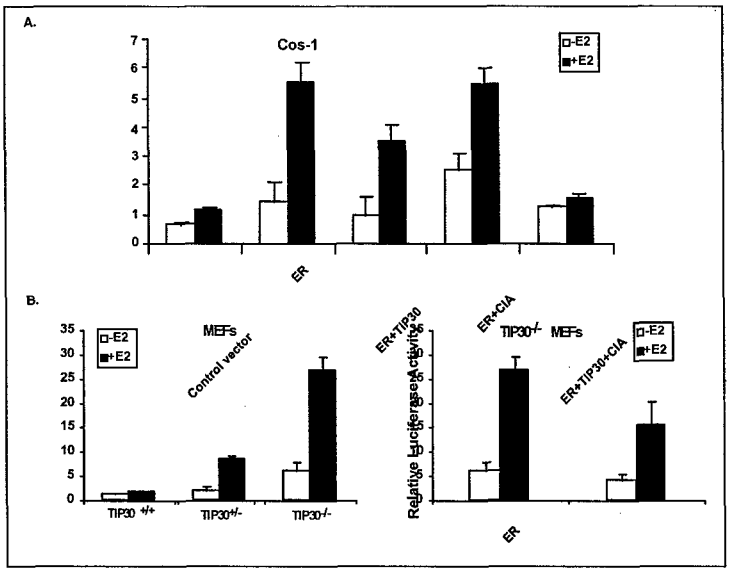
Body:

Task 1. **To determine the effects of TIP30 and CIA on expression of ERα-responsive genes, such as c-Myc and Cyclin D1 (months 1-12).**

a. Prepare various plasmid DNA for transient transfection assays (months 1-2)
b. Perform transient transfection and luciferase assays to determine the effects of TIP30 and CIA on transcription of the c-Myc and Cyclin D1 promoter (months 3-9).
c. Perform ChIP assays to determine the occupancy of TIP30 and CIA on the c-Myc and Cyclin D1 gene in vivo (months 10-16)

We have completed this task and the results from this work were included in an article (6) that was published in Journal of Biological Chemistry 2004 (attached in appendix and JBC online).

a. CIA and TIP30 act as co-regulators in ERα-mediated transcription. Transcription of the c-*myc* gene is controlled by transcription factors that interact with numerous positive and negative regulatory elements in the c-*myc* promoter regions. ERα can stimulate c-*myc* transcription by interacting with an estrogen-responsive element of the c-*myc* promoter. To test whether CIA and TIP30 function as coregulators of ERα, we performed transient-transfection experiments in Cos-1 cells with vectors expressing ERα, CIA, TIP30, and a construct containing a luciferase reporter gene controlled by the c-*myc* promoter (-2.3 kb 5' of P1 start site to +50 relative to P2 start site). We have constructed and prepared plasmid DNA that is necessary for transient transfection assays. As shown in Fig. 1A, ectopic TIP30 inhibited the E2-dependent transcriptional activity of ERα in Cos-1 cells, wereas



ectopic CIA had no significant effect on transcription. Consistent with previous observations, ectopic CIA potentiated ERα-mediated transcription on a synthetic promoter containing three ER binding sites in the presence of E2 (data not shown). Surprisingly and paradoxically, coexpression of ectopic CIA with ectopic TIP30 did not reverse, but instead further potentiated the inhibitory effect of TIP30 (4-fold) in ERα-mediated

Fig. 1. The roles of CIA and TIP30 in ERα-mediated transcription. (A) Cos-1 cells were transfected with the reporter plasmid containing the human c-*myc* promoter and vectors for expressing ER, CIA and TIP30 in the presence of 10^{-8}M E2. (B) Deletion of the TIP30 gene affects the activity of ERα. *TIP30*$^{+/+}$, *TIP30*$^{+/-}$ and *TIP30*$^{-/-}$ MEFs were transfected with the reporter plasmid containing the human c-*myc* promoter and the expression vector for ERα in the presence of either 10^{-8}M E2 or ethanol carrier (Left panel). TIP30$^{-/-}$ MEFs were cotransfected with ERα and TIP30 in the presence of either 10^{-8}M E2 or ethanol (Right panel). The luciferase activities were measured as described previously. Values shown are the mean ±S.D. from three independent experiments.

transcription. Since western blot analyses revealed that the levels of transiently-expressed ectopic TIP30 and CIA were much higher than the levels of endogenous TIP30 and CIA in Cos-1 cells (data not shown), the effects of TIP30 and CIA on ERα-mediated transcription observed here may not represent their physiological functions. Nevertheless, these results indicate that both CIA and TIP30 are important interacting factors in modulating the activity of ERα and CIA can cooperate with TIP30 to repress ERα-mediated transcription when they are overexpressed. In view of these results, especially the inhibitory effect of ectopic TIP30, we also tested whether the complete absence of TIP30 in cells affects the transcriptional activity of ERα on the c-*myc* promoter. Indeed, ERα showed a greater stimulation of the c-*myc* promoter in *Tip30*$^{-/-}$ mouse embryonic fibroblasts (MEFs) than in *Tip30*$^{+/+}$ MEFs (16-fold Fig. 1B, left panel) and *Tip30*$^{+/-}$ MEFs (3-fold lower). TIP30 loss also resulted in a 5-fold increase in estrogen-independent transcription from the *myc* prompter (Fig. 1B, left panel). This effect was due to the absence of TIP30 since expression of TIP30 in *Tip30*$^{-/-}$ cells resulted in a 42% inhibition in transcriptional activity of ERα (Fig. 1B, right panel). The finding that deletion of the *Tip30* gene elicits higher

transcription from the c-*myc* promoter indicates that TIP30 functions to repress both estrogen-independent and ERα-mediated c-*myc* transcription.

To determine whether TIP30 is able to inhibit ERα-mediated transcription on a simple promoter containing the estrogen responsive element (ERE), we used an artificial promoter containing three EREs as a reporter for transient transfection assays in $Tip30^{+/+}$, $Tip30^{+/-}$ and $Tip30^{-/-}$ MEFs (Fig. 2). Fig. 2A shows that loss of TIP30 results in an increased ER mediated transcription from this artificial promoter. We also observed cooperative inhibition of ERα-mediated transcription by ectopic expression of TIP30 and CIA on this promoter in $Tip30^{-/-}$ MEFs (Fig. 2B). This result is consistent with the observation that estrogen induces the recruitment of TIP30 and CIA to the *c-myc* promoter during the second transcription cycle and suggests that TIP30 can act through the ERE binding site.

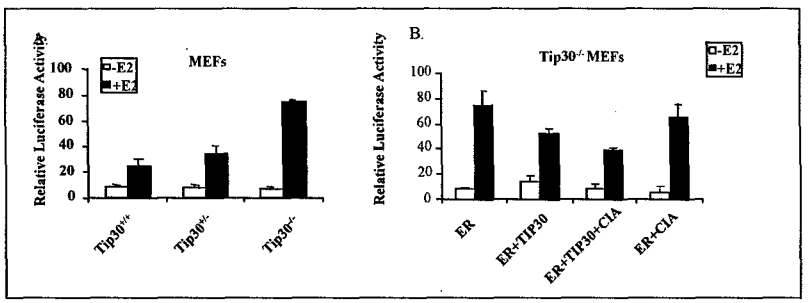


Fig. 2. TIP30 is involved in inhibition of transcription from an artificial ERE promoter. Transient transfection assays were perfomed as described in Fig. 1b and c.

b. Estrogen regulates TIP30 and CIA occupancy of the c-*myc* gene in breast cancer MCF-7 cells. Transcription of the *c-myc* gene is controlled by transcription factors that interact with numerous positive and negative regulatory elements in the *c-myc* promoter regions. ERα can stimulate *c-myc* transcription by interacting with an estrogen-responsive element of the *c-myc* promoter (8). Using chromatin immunoprecipitation (ChIP) assays, a previous study has established that a number of ERα interacting coactivators are recruited to the promoters of endogenous estrogen-responsive target genes, including *c-myc*, following estrogen treatment (8). If CIA and TIP30 are specific cofactors for ERα, they might also be associated with ERα on endogenous estrogen-responsive target genes. To test this possibility, we used the same ChIP assay (8) to determine if CIA and/or TIP30 is recruited to the promoter of the *c-myc* gene in the estrogen-dependent human breast cancer cell line MCF-7. Fig. 3B shows that, as previously reported, the anti-ERα antibody precipitated a *c-myc* promoter fragment containing P1 and P2 promoters in the presence (lane 6) but not in the absence (lane 5) of estradiol (E2). In contrast, the *c-myc* promoter was precipitated by anti-TIP30 antibody (lane 3), but much less so by anti-CIA antibody (lane 1) in the absence of E2. E2 increased the association of CIA (lane 2) but diminished the association of TIP30 (lane 4) with the same DNA region.

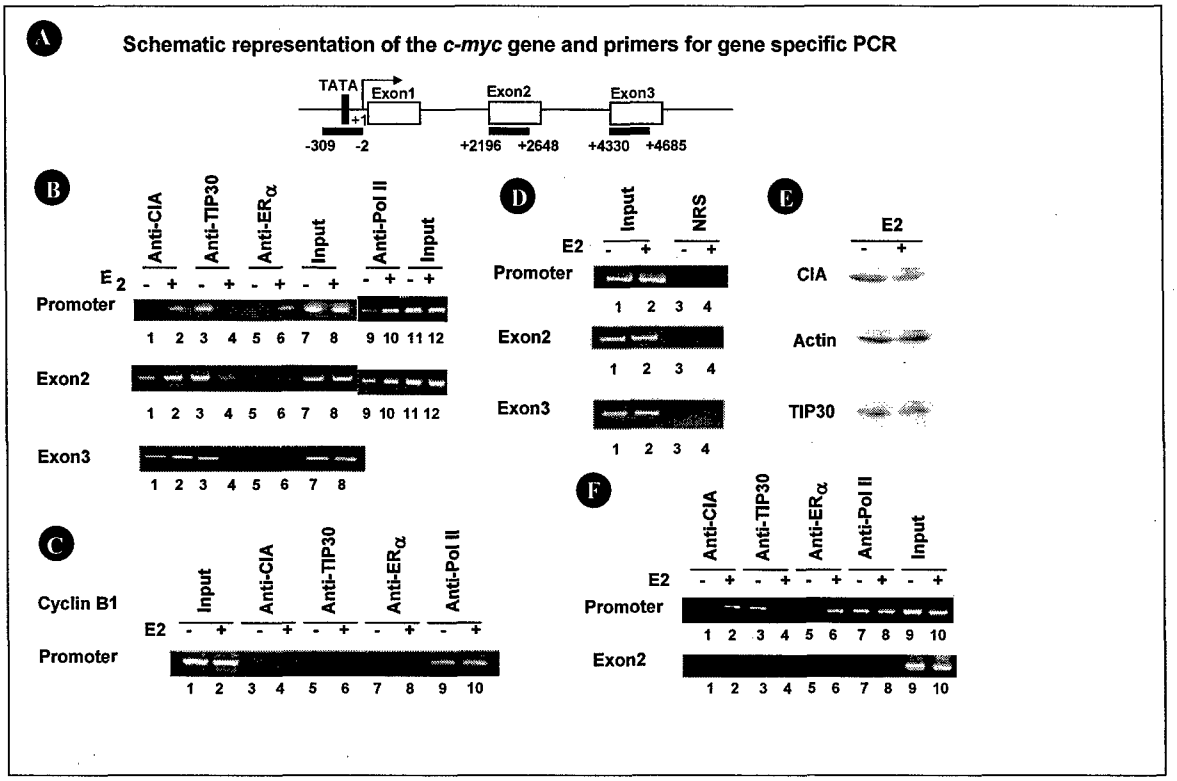


Fig. 3. Recruitment of CIA and TIP30 to the endogenous *c-myc* gene. (A) Schematic representation of the *c-myc* gene and primers for PCR. Transcription initiation site and start codon are indicated. Solid bars and the numbers indicate the positions of the primers corresponding to the regions of the *c-myc* gene. The end of *c-myc* mRNA is at the position 5190 or 5350 . (B) Assembly of CIA and TIP30 on the *c-myc* gene in the first transcription cycle upon E2 induction. MCF-7 cells were treated with 10^{-8}M E2 for 45 min. Recruitment of ERα and CIA to the promoter of the *c-myc* gene (upper panel). The 307 bp amplified DNA fragment corresponds to the region of the promoter. Association of CIA with exon 2 that is 2 kb downstream of the initiation site (middle panel) or exon 3 that is 4 kb downstream of the initiation site (Lower panel). The 452 bp or 355 bp amplified DNA fragments correspond to the region of exon 2 or exon 3, respectively. (C) ERα, TIP30 and CIA are not assembled on *Cyc B1* promoter. Primer pairs covering the –75 to +185 region (33) were used for ChIP analysis. (D) ChIP analysis with a normal rabbit pre-immune serum (NRS). (E) CIA and TIP30 protein levels in MCF-7 cells in MCF-7 cells after E2 treatment. Cell lysates were prepared from MCF-7 cells treated with or without E2 for 45 min and then analyzed by Western blotting with anti-TIP30, anti-CIA or anti-β-Actin antibodies. (F) Occupancy of TIP30 and CIA on exon 2 is inhibited by α-amanatin. ChIP analysis was performed after cells were treated with 10 μg/ml of α-amanatin for 1hr before the addition of E2.

Since previous studies demonstrated that ERα and coactivators (AIB1, p300, CBP, pCAF and TRAP220) are assembled on the promoter during pre-initiation, but subsequently released during elongation (8), we next determined whether CIA and TIP30 occupy the *c-myc* gene during elongation. Four pairs of primers covering a region (+2196 to +2628) in exon 2 and a region (+4330 to +4685) in exon 3 were used for PCR amplification of the final DNA preparations. Binding of both TIP30 and CIA to these regions was observed in the absence of E2 (Fig. 3B, lanes 1 and 3). E2 induction resulted in increased CIA binding (lanes 1 vs. 2),

but decreased TIP30 binding (lanes 3 vs. 4) to these regions. As a positive control, binding of the largest subunit of RNA polymerase II to the regions upstream and downstream of the initiation site was also observed in both the absence and presence of E2 (Fig. 2B, lanes 9 and 10). These results suggest that estrogen increases association of CIA with the coding regions of *c-myc*.

In order to assess the specificity of association of TIP30 and CIA with the *c-myc* gene, we examined whether TIP30 and CIA were assembled on the promoter of the cyclin B1 gene that is not directly regulated by ERα. As expected, RNA polymerase II is associated with the promoter (Fig. 3C, lanes 9 and 10), but ERα is not (Lanes 7 and 8). However, ERα, TIP30 and CIA were not associated with the promoter in either the presence or the absence of E2 (Lanes 3-6). In addition, non-specific antibodies from a pre-immune rabbit serum did not precipitate the DNA elements of the *c-myc* gene (Fig. 3D). The protein levels of TIP30 and CIA in MCF-7 cells are not significantly changed after E2 treatment (Fig . 3E), suggesting that E2-regulated association of TIP30 and CIA with the *c-myc* gene is not due to the influence of TIP30 and CIA expression by E2. Therefore, associations of these proteins with the *c-myc* gene are specific.

To determine whether the occupancy of the *c-myc* gene by TIP30 and CIA requires elongating RNA polymerase II in estrogen-dependent and independent transcription, MCF-7 cells were treated with α-amanitin, which specifically inhibits RNA polymerase II elongation, and then subjected to ChIP analysis. Consistent with the preceding observations, the occupancy of the *c-myc* promoter by TIP30 and CIA was not affected by α-amanitin (Fig. 3F). In contrast, the occupancy of the coding region of the *c-myc* gene by TIP30 and CIA was inhibited by α-amanitin (Fig. 3E). This result suggests that association of TIP30 and CIA with the coding regions of the *c-myc* gene depends on elongating RNA polymerase II.

Occupancy of ERα and RNA polymerase II on the promoter was previously shown to peak at 30-45 minutes in the first transcription cycle and at 120-150 minutes in the second cycle following the addition of E2 (8). We therefore sought to determine the timing of TIP30 and CIA occupancy on the promoter. As was shown previously, ERα and CBP occupancy on the promoter peaked at 30-60 min in the first transcription cycle and at 150 min in the second cycle upon E2 treatment (Fig. 3D). The results obtained with CIA were similar to those obtained with ERα and CBP. Interestingly, TIP30 was dissociated from the promoter at 60 min and re-associated with the promoter at 120-150 min in the second cycle (Fig. 3D). Binding of TIP30 and CIA to exon 2 was also observed at 150 min following E2 treatment (Fig. 3E). In contrast, much less CBP was detected to associate with exon 2 upon E2 induction.

Taken together, these results demonstrate that TIP30 is associated with the c-Myc promoter, and remains with the elongation complex in the absence of E2, while association of CIA with the promoter is less evident under these conditions. Strikingly, during the first transcription cycle, estrogen induces the recruitment of CIA to, but dissociation of TIP30 from, the c-*myc* promoter region. Unlike the other known ER coactivators, which are released during elongation, CIA shows an increased association with the transcribed region

following E2 treatment. As was observed for ERα and CBP, both CIA and TIP30 are associated with the promoter in the second cycle of estrogen-induced transcription. (Fig. 3E).

Dynamic assembly of TIP30 and CIA on the cyclin D1 promoter. We next asked whether TIP30 and CIA are recruited to the promoter of cyclin D1 gene that is also an ERα-targeting gene. With a ChIP assay, we found that CIA was also assembled on the cycD1 promoter in the presence of E2 (Fig. 4). E2 induced dissociation of TIP30 from the promoter.

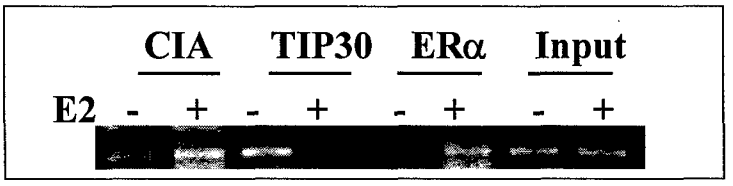


Fig. 4. ChIP assay reveals that TIP30 and CIA are dynamically assembled on the cyclin D1 promoter. ChIP assays were done as described above. A pair of primers covering ERα-binding region was used for amplification of DNA.

Task 2. **Test the effects of SERMs on TIP30 and CIA mediated transcriptional regulation of the c-Myc and the cyclin D1 genes (months 17-20).**

1. Perform transient transfection and luciferase assays to determine the effects of SERMs on TIP30 and CIA mediated transcriptional regulation of the c-Myc and cyclin D1 genes (months 17-20).
2. Perform ChIP assays to determine the effects of SERMs on recruitment of TIP30 and CIA to the c-Myc and cyclin D1 genes (months 20-24)

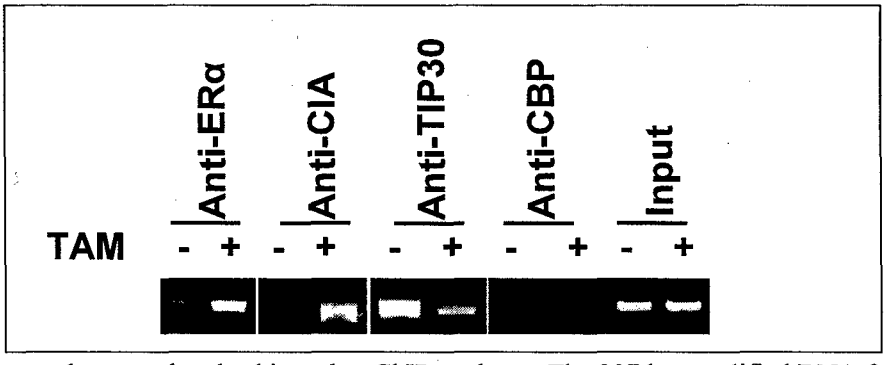


Fig. 5. TAM induces recruitment of ERα and CIA but not TIP30. MCF-7 were grown in estrogen-depleted medium for three days and treated with 10^{-8}M TAM for 45 min. Cells were harvested and subjected to ChIP analyses. The 307 bp amplified DNA fragment corresponds to the region of the promoter.

Tamoxifen (TAM) is an antagonist of E2 that inhibits ERα-mediated transcription in MCF-7 cells. This is partly because TAM-bound ERα recruits the N-CoR/SMRT co-repressors to the promoter and prevents recruitment of p160, AIB1, CBP and p300. To investigate whether TAM affects occupancy of TIP30 and CIA on the *c-myc* promoter, MCF-7 cells were treated with TAM and subjected to ChIP analyses. As expected, recruitment of ERα but not CBP, to the *c-myc* promoter was observed (Fig. 5). As was observed with E2, TAM induced association of CIA with the *c-myc* promoter but reduced TIP30 occupancy. This result suggested that CIA, but not TIP30, was present in the TAM-ERα repression complex

assembled on the promoter of the *c-myc* gene.

Since we did not detect an increase in CycD1 expression in Tip30 null mouse mammary epithelial cells, we believe that CycD1 is not a main target gene controlled by TIP30. Therefore, we decided to focus our study on Myc expression.

Task 3. **To determine the effects of TIP30 and CIA on the proliferation, motility and apoptosis in ER-negative MDA-MB-231 breast cancer cells.**

a. Establish various MDA-MB-231 breast cancer cell lines stably expressing TIP30, TIP30 mutant, CIA or CIA mutant (months 1-6)

b. Measure the growth rates of various cell lines and perform MTT assays to determine rates of DNA synthesis (months 7-12)

c. Measure the capacities of cells to migrate and invade in order to assess the ability of TIP30 and CIA to regulate motility of breast cancer cells (months 13-14).

d. Assay the sensitivity of cells to apopotsis in order to assess the ability of TIP30 and CIA to induce cell death (months 15-20)

e. Develop a series of retroviruses expressing wild type and mutant TIP30 and CIA (months 21- 28)

f. Perform viral infections and assays for growth rates and apoptosis to determine whether ectopic expression of both TIP30 and CIA in breast cancer cells could inhibit cell growth and induce apopotsis (months 28-36)

We have completed all experiments in task 3 except experiments proposed in e. In the proposal, we planed to test TIP30 mutants made artificially in vitro for their ability to induce apoptosis. Recently, we have discovered that mutations in the TIP30 gene frequently occur in ductal carcinoma patients. We have recently identified several TIP30 mutants from ductal carcinoma patients. Therefore, we have decided to test whether those mutants are able to induce apoptosis or promote cell growth.

TIP30 and CIA act synergistically to promote cell death in MDA-MB-231 cells

In order to assess the biological significance of overexpression of TIP30 and/or CIA in the growth of estrogen-independent breast cancer cells, TIP30 and CIA expression vectors were introduced into MDA-MB-231 cells. We first used TIP30 and CIA expression plasmids to make stable cell lines. We found that the efficiency of transient transfection in this cell line was very low using several commercial reagents. To efficiently introduce the expression vectors, we then constructed the pBabe-flagTIP30-puro retroviral expression vector and pBabe-CIA-puro retroviral expression vector and used 293T cells to generate replication-deficient viral particles of pBabe-puro, pBabe-flagTIP30-puro, pBabe-CIA-puro, or pBabe-flagTIP30-puro and pBabe-CIA-puro. We have made stable MDA231 cells containing expressing vectors. However, we could not detect either ectopic wild-type TIP30 or ectopic CIA proteins in those cells by Western blot analysis. However, the MDA-MB-231 cells containing mutant TIP30 expression vectors overexpress mutant TIP30 proteins. This result suggests that overexpression of TIP30 or CIA was not well tolerated by these cells. Therefore, a possibility for failure of cells stably overexpressing TIP30 or CIA is that overexpression of TIP30 or CIA may

suppress the growth of MDA-MB-231 cells through induction of apopotsis. To test this possibility, we examined the growth of MDA-MB-231 cells in a short-term expression assay by utilizing retroviruses expressing TIP30 and/or CIA for three days. At 48 hours after infection with retroviruses and antibiotic selections, the MDA-MB-231 cells were plated in low serum culture at equal density, and cells were counted after 36 hrs. We found that less than 2% live cells were found in the plates of MDA-MB-231 cells that had been infected with retroviruses expressing both TIP30 and CIA. Based on a preliminary study, we proposed that ectopic expression of TIP30 could cooperate with CIA to inhibit MDA-MB-231 cell growth. However, in this experiment, it was not clear whether a MDA-MB-231 cell ectopically expressed both TIP30 and CIA. In order to assure that a cell expressed both ectopic TIP30 and CIA, we have created a retrovirus expressing both TIP30 and CIA by constructing the TIP30 and CIA genes into the viral expression vector and inserting a ribosomal binding site (IRE) between the TIP30 and CIA genes that allows translation of both proteins from one mRNA. We examined the growth of MDA-MB-231 cells in a short-term expression assay by utilizing retroviruses expressing TIP30 and/or CIA. At 48 hours after infection with retroviruses and antibiotic selections, the MDA-MB-231 cells were plated in low serum culture at equal density. Proliferation and growth of cells were measured by MTT assay and counted every 24 hours for six days. As shown in Fig. 2, proliferation of cells was not significantly affected by the retrovirus expressing TIP30 as compared to cells infected with control viruses. In contrast, proliferation of cells was significantly inhibited by retroviruses expressing CIA and completely inhibited by retroviruses expressing both CIA and TIP30. Similarly, MDA-MB-231 cells infected with control retroviruses or TIP30-expressing viruses grow at a similar rate. This data support our hypothesis that TIP30 and CIA act synergistically to predispose estrogen-independent breast cancer cells to apoptosis.

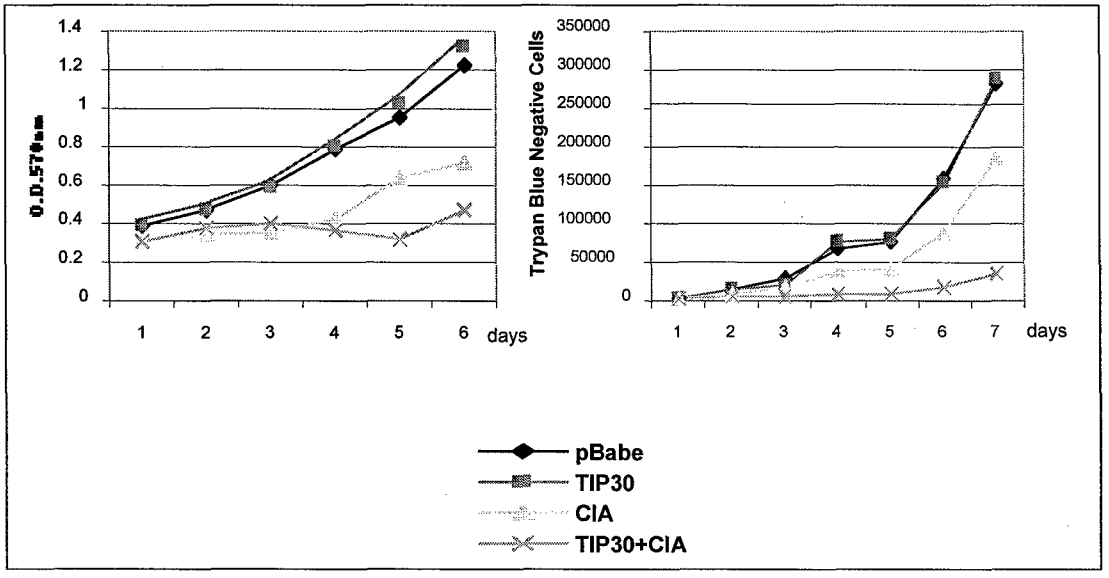


Fig 6. 293T cells were transfected with empty pBabe-puro vector, pBabe-flagTIP30-puro, pBabe-CIA-puro, or pBabe-flagTIP30-CIA puro. Virus-containing supernatants were collected 48 hours after transfection. The virus-infected MDA-MB-231 cells were

selected in complete media with puromycin for 48 hours and then seeded into the plates. At indicated time points, MTT assay was performed as described and O.D. measurements were plotted (Left panel). At indicated time points, living and dead cells were counted using trypan blue exclusion (right panel). Values shown are average numbers from three experiments.

We next investigated whether expression of TIP30 or/and CIA in MDA-MB-231 cells sensitizes the cells to cisplatin-induced apoptosis. Expression of TIP30 in cells resulted in a 72% reduction of viable cells in comparison to control vector (Fig. 7). In contrast, expression of either TIP30 or CIA alone resulted in 29% or 43% reduction of viable cells in comparison with control vector, respectively. Therefore, overexpression of TIP30 and CIA in cells predisposes them to apoptosis induced by cisplatin.

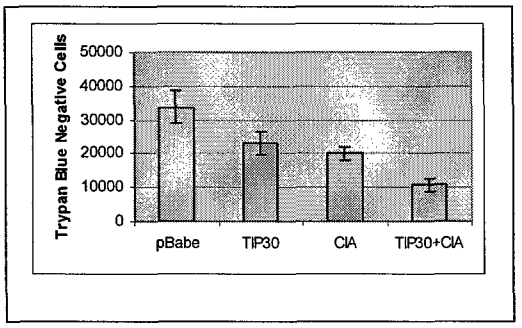


Fig. 7. MDA-MB-231 cells were infected with indicated TIP30 or/and CIA expression viruses. The virus-infected MDA-MB-231 cells were selected in complete media with puromycin for 48 hours and then the same numbers of cells seeded into the plates were treated with cisplatin in 0.1% of serum and after 24 hours, trypan blue positive cells were counted.

In summary, we have completed almost all tasks that we proposed to do in the original proposal. The results were partly published in Journal of Biological Chemistry in 2004. We have requested a non-cost extension for a year to complete the entire study.

Key Research Accomplishments:
Our data demonstate that TIP30 is a negative transcriptional regulator for ERα-mediated *c-myc* transcription. Overexpression of TIP30 and CIA in estrogen-independent breast cancer cells robustly induce apoptosis. TIP30 and CIA represent new targets for developing anti-breast cancer therapy.

Reportable outcomes:
1. Part of this work was published in Journal of Biological Chemistry, 2004, 279 (26), 27781-27789.

2. Part of this work was presented as a poster at the 2003 AACR Meeting on Breast Cancer. Abstract Title: A tumor suppressor TIP30 regulates c-MYC transcription and mammogenesis.

3. Part of this work was presented as a poster at the AACR Special Conference in Cancer Research. Hyatt Regency Huntington Beach Resort and Spa. Huntington Beach, CA. October 8-12, 2003

A42 Chao Jiang, Mitushiro Ito, Kay-Uwe Wagner, Kyung-Ran Park, Kristy Krumm, Jill

Pecha, Robert G. Roeder and Hua Xiao. TIP30 Interacts with an ERα-interacting Coactivator CIA and Regulates c-myc Transcription and Mammogenesis.

4. Part of this work was presented by Chao Jiang at the AACR 95th Annual Meeting,Orlando, FL. March 27-31, 2004. 5557. The title of her talk was "TIP30 acts as a tumor suppressor in hepatocarcinogenesis".

5. The results from this work were included in the preliminary data section for a five-year NIH grant application entitled "Role of a tumor suppressor in tumorigenesis" that has been funded for five years by NIH.

6. Part of this work will be presented as a poster by Hua Xiao at Era of Hopes 2005– Department of Defense Breast Cancer Research Meeting in June 8-11, 2005. The title of the poster is 'TIP30, a newly identified tumor suppressor in breast carcinogenesis".

Conclusions:

We demonstrate that a tumor suppressor TIP30 and an ER-interacting coactivator CIA are dynamically associated with transcription initiation and elongation complexes in response to estrogen. TIP30 overexpression represses ERα-mediated c-*myc* transcription, whereas TIP30 deficiency enhances *c-myc* transcription in the absence and presence of estrogen. Ectopic CIA cooperates with TIP30 to repress ERα-mediated c-*myc* transcription. In addition, overexpression of TIP30 and CIA in estrogen-independent breast cancer cells suppresses cell growth and induces apoptosis. We suggest that TIP30 and CIA act as negative regulators to control transcription of *c-myc* and cyclin D1. Our data provides a new pathway for TIP30-mediated tumor suppression and sets a stage to study the mechanisms in which this cofactor regulates expression of genes that play important roles in tumorigenesis. Thus, human Tip30 may represent a new valuable diagnostic and therapeutic target for breast cancer.

References:

1. McKenna NJ, Lanz RB, O'Malley BW. (1999) Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev.* 3, 321-44.
2. Sommer S, Fuqua SA. (2001). Estrogen receptor and breast cancer. Semin Cancer Biol *11,* 339-52.
3. Xiao, H, Tao, Y, Greenblatt, J., Roeder, R.G. (1998). A cofactor, TIP30, specifically enhances HIV-1 Tat-activated transcription. Proc. Natl. Acad. Sci .USA. *95,* 13519-24
4. Shtivelman, E. (1997). A link between metastasis and resistance to apoptosis of variant small cell lung carcinoma. Oncogene *14,* 2167-73.
5. Ito M., Jiang C., Krumm ,K ., Zhang, X., Pecha, J., Zhao, J., Guo Y. Roeder RG., and Xiao H. (2003) TIP30 deficiency increases susceptibility to tumorigenesis. Cancer Research, 63: 8763-7.

6. Jiang, C., Ito M., Piening, V., Bruck, K., Roeder, R.G. and Xiao H. (2004) TIP30 interacts with an ERα-interacting coactivator, CIA and regulates *c-myc* transcription. J. Biol. Chem. 279 (26), 27781-27789.
7. Sauve, F., McBroom, L.D., Gallant, J., Moraitis, A.N., Labrie, F., Giguere, V. (2001). CIA, a novel estrogen receptor coactivator with a bifunctional nuclear receptor interacting determinant. Mol Cell Biol. J. *1,* 343-53.
8. Shang, Y., Hu, X., DiRenzo, J., Lazar, M.A., Brown, M. (2000). Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell *103,* 843-52.